



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

June 18, 2007

**MEMORANDUM**

**SUBJECT:** Review of Activated Sludge, Respiration Inhibition Test Data for  
Octhilinone (99.2% a.i.) Reregistration Eligibility Decision (RED)  
Document

**Reregistration Case No.:** 2475

**DP Barcode:** 339813

**MRID No.:** 471070-08

**FROM:** Srinivas Gowda, Microbiologist/Chemist *Srinivas Gowda*  
Risk Assessment and Science Support Branch (RASSB)  
Antimicrobials Division (7510P)

**TO:** Diane Isbell, Team Leader  
Kathryn Jakob, CRM  
Regulatory Management Branch II  
Antimicrobials Division (7510P)

**THRU:** Siroos Mostaghimi, Team Leader, Team one *Siroos Mostaghimi*  
Risk Assessment and Science Support Branch (RASSB)  
Antimicrobials Division (7510P)

Norman Cook, Branch Chief *Norman Cook*  
Risk Assessment and Science Support Branch (RASSB)  
Antimicrobials Division (7510P)

<u>Chemical Name</u>	<u>PC Code</u>	<u>CAS#</u>	<u>Common Names</u>
2-Octyl-3 (2H)-isothiazolone	099901	26530-20-1	Octhilinone

## **INTRODUCTION:**

The Registrant, Rohm and Hass Company, has submitted Activated Sludge, Respiration Inhibition test data for Othilinone (99.2% a.i.; PC Code 099901) Reregistration Eligibility Decision (RED) Document. The submitted Activated Sludge, Respiration Inhibition study has undergone review by Srinivas Gowda of Antimicrobials Division's Risk Assessment and Science Support Branch. The registrant used the OPPTS 850-6800 & OECD 209 Guideline to meet the Activated Sludge, Respiration Inhibition study data requirements for Othilinone.

## **BACKGROUND:**

The submitted Activated Sludge, Respiration Inhibition study was conducted to assess the effect of Othilinone (PC Code 099901) on sewage micro-organisms by measuring the respiration rate under defined conditions in the presence of different concentrations of the test substance.

## **CITATION:**

Othilinone – Activated Sludge Respiration Inhibition by Amy Ouellette, Springborn Laboratories Inc. (SLI), Environmental Sciences Division, 790 Main Street, Wareham, Massachusetts 02571-1075, Rohm and Haas Report No. 95RC-060, SLI Report # 95-9-6086, and SLI Study # 86.0695.6188.790, dated November 1, 1995 (MRID No. 471070-08).

**DATA EVALUATION:** Attached.

## **CONCLUSIONS:**

The test results are valid and met the criteria for validity specified in the OPPTS 850-6800 & OECD 209 guidelines as follows:

1. For Othilinone, respiration was inhibited by 11, 28, 30, 33, and 91% compared to controls at 4.0, 10, 25, 63, and 125 mg/L, respectively ( $EC_{50}$  47 mg/L).
2. For reference material (3,5-dichlorophenol, a known inhibitor of respiration), respiration was inhibited by 22, 65, and 80% compared to controls in the 5.0, 15, and 30 mg/L groups ( $EC_{50}$  11 mg/L). The  $EC_{50}$  of the reference material (3,5-dichlorophenol) of 11 mg/L is within the accepted range of 5 to 30 mg/L.
3. The control respiration rates were within 15% of each other.

## **RECOMMENDATIONS:**

The submitted Activated Sludge, Respiration Inhibition study by the OECD 209/OPPTS 850.6800 guidelines is scientifically valid and acceptable. The  $EC_{50}$  of Othilinone is 47 mg/L. RASSB recommends that the submitted Activated Sludge, Respiration Inhibition study be accepted in support of Reregistration of the active ingredient, Othilinone (99.2% a.i.).

## ACTIVATED SLUDGE, RESPIRATION INHIBITION TEST

### DATA EVALUATION REPORT

**PRODUCT FORMULATION:** Octhilinone (99.2% a.i.)

**ACTIVE INGREDIENTS:** Mixture of: 2-N-octyl-4-isothiazolin-3-one (>96%), chlorobenzene (<1%), and related reaction products (<3%).

**BACKGROUND:** The study was submitted to assess the effect of Octhilinone (PC Code 099901) on microorganisms by measuring the respiration rate under defined conditions in the presence of different concentrations of the test substance. The study was conducted according to the OECD 209/OPPTS 850.6800.

#### CITATION

**Author:** Amy Ouellette  
**Title:** Octhilinone – Activated Sludge Respiration Inhibition  
**Study Completion Date:** November 1, 1995  
**Laboratory:** Springborn Laboratories Inc. (SLI)  
Environmental Sciences Division  
790 Main Street  
Wareham, Massachusetts 02571-1075  
**Sponsor:** Rohm and Haas Company  
727 Norristown Road  
P.O. Box 0904  
Spring House, Pennsylvania 19477-0904  
**Laboratory Report ID:** Rohm and Haas Report No. 95RC-060  
SLI Report # 95-9-6086  
SLI Study # 86.0695.6188.790  
**MRID No.:** 471070-08

**OPPTS/OECD Guideline Nos.:** OPPTS 850.6800 & OECD 209.

#### 1.0 EXECUTIVE SUMMARY.

The purpose of this study was to assess the effect of Octhilinone on microorganisms by measuring the respiration rate under defined conditions in the presence of different concentrations of the test material after a 3-hour time period. The study was conducted according to the procedures outlined in the Organization for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals, Guideline 209, Activated Sludge, Respiration Inhibition Test.

Two controls (i.e., no chemicals added), five test material concentrations (i.e., Octhilinone at 4.0, 10, 25, 63, or 160 mg/L), and three reference material concentrations (i.e., 3,5-dichlorophenol at 5, 15, or 30 mg/L) were mixed with 16 mL of synthetic sewage feed, 284 mL water (including appropriate concentration of the test substance), and 200 mL of microbial



inoculum in 1000 mL beakers to begin the contact period. After three hours of aeration, the contents of each beaker were poured into a BOD bottle and the dissolved oxygen concentration of each reaction mixture was recorded over a period of 10 minutes.

The oxygen consumption rate was calculated for each test vessel (control, test substance, or reference material) by plotting dissolved oxygen measurements versus time. Using the linear portions of the curve, between  $t_1 = 2$  min and  $t_2 = 10$  min, the oxygen consumption rate was calculated as the difference in oxygen concentration at 2 minutes and the oxygen concentration at 10 minutes divided by the difference in time (i.e., 8 minutes). The percent inhibition was calculated at each concentration of test substance and reference substance using the mean of the two controls.  $EC_{50}$  values were determined by fitting respiration data (% inhibition) versus log-transformed concentration data to a best-fit linear regression curve based on least squares analysis.

For Octhilinone, respiration was inhibited by 11, 28, 30, 33, and 91% compared to controls at 4.0, 10, 25, 63, and 125 mg/L, respectively. Linear regression resulted in an  $EC_{50}$  value of 47 mg/L. For 3,5-dichlorophenol, respiration was inhibited by 22, 65, and 80% compared to controls in the 5.0, 15, and 30 mg/L groups. Linear regression resulted in an  $EC_{50}$  value of 11 mg/L.

#### Compliance:

Signed and dated Data Confidentiality, Good Laboratory Practice, and Quality Assurance Statements were provided. The study sponsor waived claims of confidentiality within the scope of FIFRA Sections 10(d)(1)(A), (B), or (C). The Study Report indicated that the study was conducted under EPA Good Laboratory Practice Standards (40 CFR Part 160) without exceptions.

#### Deviations:

The study was reviewed using both the OECD Guideline 209 and the EPA Ecological Effects Test Guideline 850.6800. No study deficiencies were noted.

The reviewer's verified respiration rates and percent inhibition for the test substance and reference material, using the provided equations, and the verified results were the same as those presented in the study report. Additionally, the reviewers plotted the log of the concentrations against the percent inhibitions for Octhilinone and for 3,5-dichlorophenol. The equations resulting from these verifications yielded similar slopes and y-intercepts when compared to those presented in the study report. Finally, the reviewer's calculated  $EC_{50}$  values from the equations presented in the study report and found comparable values to those presented in the study report.

## **2.0 METHODS**

### **2.1 Study Design**

The purpose of this study was to determine the 3-hour median effective concentration ( $EC_{50}$ ) of the test substance using activated sludge. The study was conducted according to the

procedures outlined in the Organization for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals, Guideline 209, Activated Sludge, Respiration Inhibition Test.

This study was conducted according to the Springborn protocol entitled "Octhilinone Activated Sludge Respiration Inhibition Test (OECD Guideline 209)", Springborn Laboratories Protocol No. 040694/OECD 209 FDA and Protocol Amendments 1, 2, and 3 dated June 26, July 11, and September 29, 1995, respectively, from Rohm and Haas Protocol No. 95P-060.

## **2.2    Test Substance**

Chemical (purity):	Octhilinone is a mixture of 2-N-octyl-4-isothiazolin-3-one (99.2%), chlorobenzene (<1%), and related reaction products (<3%).
Description:	Amber liquid
CAS #s:	26530-20-1 (2-N-octyl-4-isothiazolin-3-one)
Lot #:	3728
Water solubility:	480 ppm
Vapor Pressure:	0.00036 mm Hg at 45°C/113°F Isothiazolone

The test material was supplied by the Sponsor. Prior to test initiation, a 100 mg/L primary stock solution was prepared by diluting 0.0505 g of the test material to volume with ASTM type II water in a 500 mL volumetric flask.

## **2.3    Reference Material**

Chemical (purity)	3,5-dichlorophenol (97.0%)
Description:	White powder
CAS#:	591-35-5
Lot #:	00410KZ

The same toxicity test was performed using the reference material, 3,5-dichlorophenol (Aldrich Chemical Co. Inc., Milwaukee, Wisconsin) instead of the test substance in order to act as a positive control to check the sensitivity of the activated sludge. The stock solution of 3,5-dichlorophenol was prepared by dissolving 0.5156 g of 3,5-dichlorophenol in 10 mL 1N NaOH, diluting the mixture with 30 mL with Reagent-grade water, slowly adding 1N H<sub>2</sub>SO<sub>4</sub> (approximately 8 mL) to the point of incipient precipitation while stirring, and diluting the mixture to 1 liter. The pH was 7.4.

## **2.4    Test Inoculum**

Prior to test initiation, 6 L of activated sludge was obtained from the Wareham Wastewater Treatment Plant (WWTP), which treats domestic sewage. Before use, the sludge solution was allowed to settle for approximately 30 minutes, and the resulting supernatant was poured off. Then the sludge was centrifuged at 1000 rpm for 10 minutes, and the resulting supernatant was poured off. The sludge was washed and centrifuged two additional times. A small amount of the washed sludge was weighed and dried to calculate the quantity of wet sludge required to be suspended in water to obtain an activated sludge containing a solid level of 4 g/L



( $\pm 10\%$ ).

## **2.5 Range-finding Study**

A range-finding test using nominal concentrations of 0.10, 1.0, 0, 100, or 1000 mg/L was performed to determine the concentration selection for the definitive toxicity test. According to the study report, the respiration inhibition ranged from 0-98%. Based on these results, concentrations of 4.0, 10, 25, 63, and 160 mg/L were selected for use in the definitive study.

## **2.6 Definitive Study**

A 100 mg/L stock solution of the test substance was diluted with water to create five nominal concentrations of the test substance, spaced by a dilution factor of approximately 2.5 (4.0, 10, 25, 63, and 160 mg/L). Test concentrations were corrected for the purity of the test substance. A co-solvent was not used in the dilutions. The test was conducted under static conditions for 3 hours at  $20 \pm 2^\circ\text{C}$ . At Time-0 for each test vessel, 16 mL of synthetic sewage feed was brought up to 300 mL in dilution water containing the appropriate concentration of test substance. The test substance stock solution was used for the 4.0, 10, and 25 mg/L concentrations, whereas raw material was added directly for the 63 and 160 mg/L concentrations. Microbial inoculum (200 mL) was added for a total of 500 mL. Test vessels were established at 15-minute intervals. The first and last vessels established were controls.

The reference material sludge mixtures were prepared at nominal concentrations of 5.0, 15, and 30 mg/L in 15-minute intervals by adding the appropriate volumes of 3,5-dichlorophenol to the sludge mixture.

During the contact period (the time the microorganisms were in contact with the test substance or reference material prior to the measurement of oxygen consumption, i.e., 3 hours), the solutions were aerated at approximately 1 L/min with room air via an oil-free pump and silicone tubing attached to a Pasteur pipet. After 3 hours of aeration, each sludge mixture was poured into a 500-mL BOD bottle fitted with a Neoprene® stopper through which a YSI Model 57 dissolved oxygen monitor was placed. The mixtures were stirred continuously, and dissolved oxygen concentration was measured every minute for 10 minutes. Solution pH was recorded using a LaMotte Model HA pH meter. Temperature of the laboratory was maintained at  $22^\circ\text{C}$ .

The definitive respiration inhibition test was conducted for 3 hours. At Time-0, the first control ( $C_1$ ) was prepared by adding 16 mL of synthetic sewage feed to a 500 mL graduated cylinder and then bringing this up to a 300-mL volume with water. Inoculum (200 mL) was added to the cylinder for a total of 500 mL. This mixture was then added to a 1-L beaker, and aeration was initiated at approximately 1 L/minute. Test substance test systems were initiated at 15-minute intervals in a similar manner. Fifteen minutes after the last test substance system was prepared, the second control ( $C_2$ ) was prepared in the same manner as  $C_1$ .

## **2.7 Calculations**

The oxygen consumption rate was calculated for each test vessel (control, test substance, or reference material) by plotting dissolved oxygen measurements versus time. Using the linear

portions of the curve, between  $t_1 = 2$  min and  $t_2 = 10$  min, the oxygen consumption rate was calculated as the difference in oxygen concentration at 2 minutes and the oxygen concentration at 10 minutes divided by the difference in time (i.e., 8 minutes) as follows:

$$\text{Oxygen consumption (mg O}_2\text{ / L} \cdot \text{min)} = (\text{mg O}_2\text{ / L at } t_1 - \text{mg O}_2\text{ / L at } t_2) / (t_2 - t_1)$$

The percent inhibition was calculated at each concentration of test substance and reference substance, according to the following equation:

$$\% \text{ inhibition} = (1 - \{2R_s / [R_{c1} + R_{c2}]\}) \times 100$$

Where:  $R_s$  = oxygen consumption rate at specified concentration of test material

$R_{c1}$  = oxygen consumption rate, Control  $C_1$

$R_{c2}$  = oxygen consumption rate, Control  $C_2$

The nominal concentrations of Othilinone or 3,5-dichlorophenol and the corresponding measurements of oxygen consumption were used to estimate a median effect concentration ( $EC_{50}$ ). The  $EC_{80}$ ,  $EC_{50}$ , and  $EC_{20}$  are the estimated concentrations of the test substance or reference substance which inhibit the respiration of the microorganisms present in activated sludge by 80, 50, and 20%, respectively, compared to the respiration in the controls.  $EC_{50}$  values were determined by fitting respiration data (% inhibition) versus log-transformed concentration data to a best-fit linear regression curve based on least squares analysis. This regression equation was then used to calculate an  $EC_{50}$  value, using inverse prediction (i.e., a percent inhibition value of 50 was substituted into the equation). If no concentration tested caused a respiration inhibition of  $\geq 50\%$ , the  $EC_{50}$  was empirically estimated to be greater than the highest concentration tested.

### 3.0 RESULTS

At test termination, the respiration rate for each of the two control vessels was measured at 0.700 and 0.650 mg  $O_2$ /L $\cdot$ min, which were comparable (i.e., the difference between these two control values was within 7.4% of their mean). For the vessels treated with Othilinone, results for dissolved oxygen concentration, oxygen consumption, and percent inhibition are presented in Table 1, and dissolved oxygen concentration versus time is presented in Figure 1 in the Appendix of this DER. Mean oxygen consumption values were decreased in a concentration-dependent manner with values of 0.600, 0.488, 0.475, 0.450, or 0.0625 mg  $O_2$ /L $\cdot$ min in the vessels treated with 4.0, 10, 25, 63, or 160 mg/L Othilinone, respectively. Thus, respiration was inhibited in a concentration-dependent manner compared to controls at 4.0 mg/L (11%), 10 mg/L (28%), 25 mg/L (30%), 63 mg/L (33), and 125 mg/L (91%). Linear regression resulted in an  $EC_{50}$  value of 47 mg/L a.i. (Figure 2).



**Table 1. Dissolved oxygen concentration, oxygen consumption, and percent inhibition for the Oclothilone treated sludge mixtures during the activated sludge respiration inhibition test. <sup>a</sup>**

test.

Time (min)	Nominal concentration (mg/L)						
	Control <sub>1</sub>	Control <sub>2</sub>	4.0	10	25	63	160
1	7.0	6.4	6.9	7.1	7.4	7.5	8.5
2	6.2	5.7	6.1	6.3	6.7	6.8	8.4
3	5.4	5.0	5.4	5.8	6.1	6.3	8.3
4	4.7	4.3	4.8	5.2	5.7	5.8	8.3
5	4.0	3.6	4.1	4.7	5.2	5.4	8.2
6	3.3	2.9	3.7	4.2	4.7	5.0	8.1
7	2.6	2.2	3.1	3.8	4.3	4.5	8.1
8	1.9	1.5	2.5	3.3	3.8	4.1	8.0
9	1.2	0.9	1.8	2.9	3.4	3.6	8.0
10	0.6	0.5	1.3	2.4	2.9	3.2	7.9
O <sub>2</sub> consumption (mg O <sub>2</sub> /L•min) <sup>b</sup>	0.700	0.650	0.600	0.488	0.475	0.450	0.0625
% inhibition <sup>c</sup>	NA	NA	11	28	30	33	91

a Data were obtained from Table 1 on page 20 of the study report.

b Oxygen consumption rate equal to the dissolved oxygen concentration at Time 2 – the dissolved oxygen concentration at Time 10 divided by the difference in minutes.

c Percent inhibition is calculated by dividing the oxygen consumption for a given concentration by the average of the two controls, subtracting this quotient from 1, and multiplying this difference by 100.

NA Not applicable

For the vessels treated with 3,5-dichlorophenol, results for dissolved oxygen concentration, oxygen consumption, and percent inhibition are presented in Table 2, and dissolved oxygen concentration versus time is presented in Figure 3 in the Appendix of this DER. Mean oxygen consumption values were decreased in a concentration-dependent manner with values of 0.525, 0.238, or 0.138 mg O<sub>2</sub>/L•min in the vessels treated with 5.0, 15, or 30 mg/L 3,5-dichlorophenol, respectively. Thus, respiration was inhibited in a concentration-dependent manner compared to controls at 5.0 mg/L (22%), 15 mg/L (65%), and 30 mg/L (80%). Linear regression resulted in an EC<sub>50</sub> value of 11 mg/L a.i. (Figure 4).



**Table 2. Dissolved oxygen concentration, oxygen consumption, and percent inhibition for the 3.5-Dichlorophenol treated sludge mixtures during the activated sludge respiration inhibition test. <sup>a</sup>**

Time (min)	Nominal concentration (mg/L)				
	Control <sub>1</sub>	Control <sub>2</sub>	5.0	15	30
1	7.0	6.4	7.0	8.0	8.4
2	6.2	5.7	6.4	7.8	8.1
3	5.4	5.0	5.9	7.5	8.0
4	4.7	4.3	5.4	7.3	7.9
5	4.0	3.6	4.8	7.1	7.7
6	3.3	2.9	4.3	6.8	7.5
7	2.6	2.2	3.8	6.6	7.4
8	1.9	1.5	3.3	6.3	7.3
9	1.2	0.9	2.8	6.1	7.1
10	0.6	0.5	2.2	5.9	7.0
O <sub>2</sub> consumption (mg O <sub>2</sub> /L•min) <sup>b</sup>	0.700	0.650	0.525	0.238	0.138
% inhibition <sup>c</sup>	NA	NA	22	65	80

a Data were obtained from Table 2 on page 21 of the study report.

b Oxygen consumption rate equal to the dissolved oxygen concentration at Time 2 – the dissolved oxygen concentration at Time 10 divided by the difference in minutes

c Percent inhibition is calculated by dividing the oxygen consumption for a given concentration by the average of the two controls, subtracting this quotient from 1, and multiplying this difference by 100.

NA Not applicable

#### 4.0 VERIFICATION OF RESULTS

The reviewer's verified respiration rates and percent inhibition for the test substance and reference material, using the provided equations, and the verified results were the same as those presented in the study report. Additionally, the reviewers plotted the log of the concentrations against the percent inhibitions for Othilinone and for 3.5-dichlorophenol. The equations resulting from these verifications yielded similar slopes and y-intercepts when compared to those presented in the study report. Finally, the reviewer's calculated EC<sub>50</sub> values from the equations presented in the study report and found comparable values to those presented in the study report.

## **5.0 DISCUSSION**

For Octhilineone, respiration was inhibited by 11, 28, 30, 33, and 91% compared to controls at 4.0, 10, 25, 63, and 125 mg/L, respectively. Linear regression resulted in an EC<sub>50</sub> value of 47 mg/L. For 3,5-dichlorophenol, respiration was inhibited by 22, 65, and 80% compared to controls in the 5.0, 15, and 30 mg/L groups. Linear regression resulted in an EC<sub>50</sub> value of 11 mg/L.

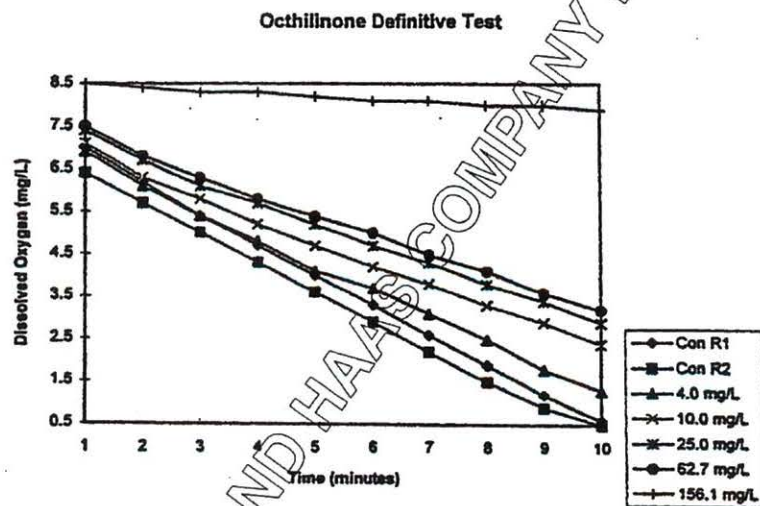
## **6.0 COMMENTS**

The study was reviewed using both the OECD Guideline 209 and the EPA Ecological Effects Test Guideline 850.6800. The test results met the criteria for validity specified in the guidelines as follows: (1) the EC<sub>50</sub> of the reference material of 11 mg/L is within the accepted range of 5 to 30 mg/L; and (2) the control respiration rates were comparable (i.e., within 15%). No study deficiencies were noted.



## APPENDIX

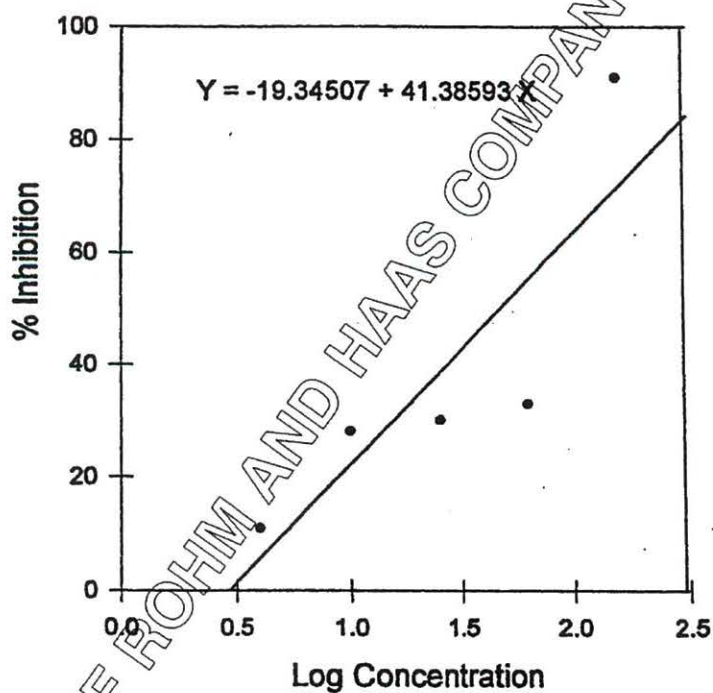
Figure 1. Dissolved oxygen concentrations versus time for Octhilineone determined during the activated sludge respiration inhibition test.



Springborn Laboratories, Inc.

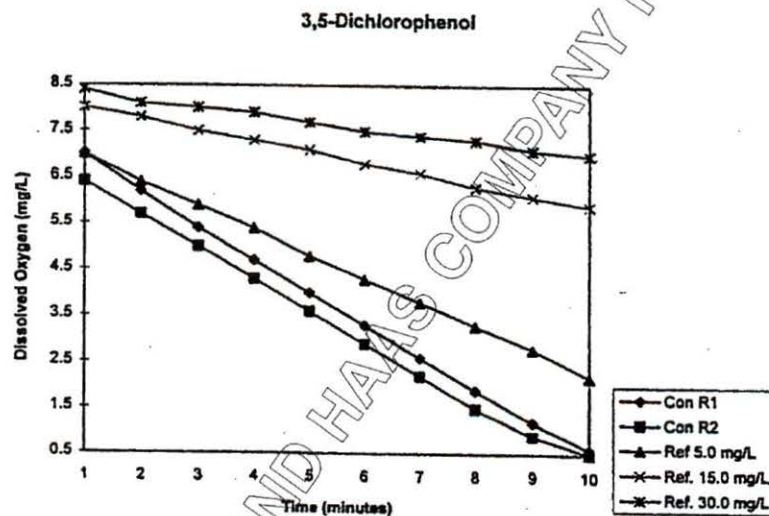


**Figure 2.** The respiration inhibition curve determined during the activated sludge respiration inhibition test with Octhilinone.



Springborn Laboratories, Inc.

Figure 3. Dissolved oxygen concentrations versus time for 3,5-dichlorophenol determined during the activated sludge respiration inhibition test with Ochtillinone.

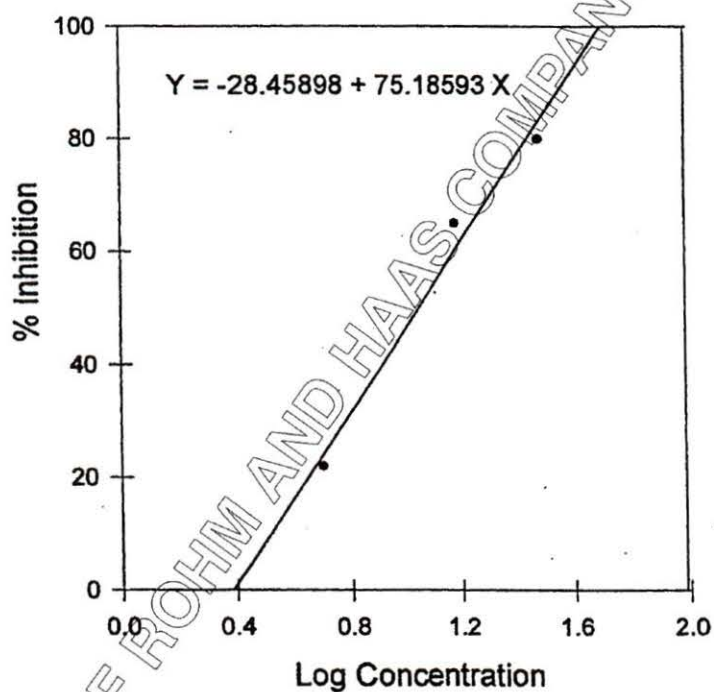


Springborn Laboratories, Inc.

FORM 4685 REV. 10/78



Figure 4. The respiration inhibition curve for 3,5-dichlorophenol determined during the activated sludge respiration inhibition test with Octhlinone.



Springborn Laboratories, Inc.

FORM 4685 REV. 10/78

# DATA PACKAGE BEAN SHEET

Date: 29-Jun-2007

Page 1 of 2

Decision #: 376412

DP #: (339813)

NON PRIA

Parent DP#: 337620

## \*\*\* Registration Information \*\*\*

Registration: RR-099901-26993 - Oclothilnone RED

Company: -

Risk Manager: RM 32 - Robert Brennis - (703) 308-6264 Room# CM-2 308G

Risk Manager Reviewer: Norman Cook NCOOK

Sent Date:

Calculated Due Date:

Edited Due Date:

Type of Registration: Project

Action Desc:

Ingredients: 099901, Oclothilnone

## \*\*\* Data Package Information \*\*\*

Expedite: Yes ☒ No

Date Sent: 10-May-2007

Due Back:

DP Ingredient: 099901, Oclothilnone

DP Title:

CSF Included: Yes ☒ No

Label Included: Yes ☒ No

Parent DP #: 337620

Assigned To

Date In

Date Out

Organization: AD / RASSB

10-May-2007

29-Jun-2007

Last Possible Science Due Date:

Team Name: RASSB1

10-May-2007

29-Jun-2007

Science Due Date:

Reviewer Name: Gowda, Srinivas

10-May-2007

29-Jun-2007

Sub Data Package Due Date:

Contractor Name:

## \*\*\* Studies Sent for Review \*\*\*

No Studies

## \*\*\* Additional Data Package for this Decision \*\*\*

Printed on Page 2

## \*\*\* Data Package Instructions \*\*\*

Sub-bean for review of activated sludge respiration inhibition test. NCook